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## (54) TREATING NIDDM WITH RXR AGONISTS

BEHANDLUNG VON NIDDM (NICHTINSULINABHÄNGIGE DIABETES MELLITUS) MIT RXR AGONISTEN

TRAITEMENT DE DIABETES NON INSULINO-DEPENDANTS A L'AIDE D'AGONISTES DE RXR

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## Description

### FIELD OF THE INVENTION

**[0001]** This invention relates to pharmaceutical compounds for treating diabetes and related symptoms.

### BACKGROUND OF THE INVENTION

**[0002]** Non-insulin-dependent diabetes mellitus (NIDDM, type II diabetes) is characterized by abnormalities in insulin secretion and insulin action. NIDDM constitutes 90-95% of the approximately 6 million diagnosed diabetics in the United States. NIDDM is characterized by hyperglycemia, the result of insulin resistance in peripheral tissues (skeletal muscle and adipose tissue), where insulin-stimulated uptake/utilization of glucose is blunted, and in liver, where insulin suppression of glucose output is insufficient. These impairments in insulin action play an important role in the development of elevated fasting blood glucose and glucose intolerance.

**[0003]** Diet and exercise are first-line therapy for NIDDM patients. NIDDM patients also take oral hypoglycemic drugs to control blood glucose levels. The most widely used hypoglycemic agents are various formulations of insulin and sulfonylureas. A major drawback with these therapies is the occurrence of potentially life-threatening hypoglycemia due to hyperinsulinemia.

**[0004]** The hyperinsulinemia that can occur with these therapies is also associated with an elevated risk of cardiovascular disease, a major killer of diabetics. Therefore, a need exists for antidiabetic drugs which do not increase circulating insulin concentrations.

**[0005]** A new class of compounds, thiazolidinediones, have been documented to effect antihyperglycemic activity by increasing insulin action rather than by promoting insulin secretion. Thiazolidinediones ameliorate insulin resistance and normalize plasma glucose and insulin (where elevated) without causing a hypoglycemic state, even at very high doses. The thiazolidinedione insulin sensitizers, e.g., ciglitazone, englitazone, pioglitazone, BRL 49653 (5-[[4-[2-(methyl-2-pyridinylamino)ethoxy]phenyl]methyl]-2,4-thiazolidinedione), and troglitazone, enhance insulin-mediated suppression of hepatic glucose output and insulin-stimulated glucose uptake and utilization by adipose tissue. Thiazolidinediones also change glucose transporter (e.g. Glut 4) expression to contribute to increased insulin responsiveness.

### SUMMARY OF THE INVENTION

**[0006]** Applicant has found that RXR agonists mimic or enhance the antidiabetic effects of thiazolidinedione compounds. RXR agonists activate the transcriptional activity of RXR/PPARγ heterodimers, increase insulin stimulated glucose uptake, lower the level of triglyceride, suppress the level of insulin, and increase the level of HDL cholesterol. Two RXR agonists have been shown to lower glucose, triglycerides and insulin levels in two established animal models of NIDDM, i.e. the *ob/ob* and *db/db* mice. Therefore, RXR agonists may be used as insulin sensitizers or insulin mimetics in the treatment of NIDDM and related symptoms. Therefore, in a first aspect, the present invention refers to the use of a Retinoid X Receptor (RXR) agonist in the manufacture of a pharmaceutical composition for treating a host having non-insulin-dependent diabetes mellitus (NIDDM) wherein said composition further comprises a peroxisome proliferator-activated receptor γ (PPARγ) agonist, said PPARγ agonist being a thiazolidinedione compound, wherein said RXR agonist is 2-[1-3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-cyclopropyl]-pyridine-5-carboxylic acid (LG 100268) or 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)ethenyl]benzoic acid (LGD 1069).

In a second aspect, the present invention refers to a pharmaceutical composition for the treatment of NIDDM comprising (a) an RXR agonist; (b) a PPARγ agonist; and (c) a pharmaceutically acceptable carrier, wherein said PPARγ agonist is a thiazolidinedione compound, and wherein said RXR agonist is selected from the group consisting of LG 100268, LGD 1069 or 9-cis retinoic acid. Said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120,744, englitazone, AD 5075 and darglitazone.

In a third aspect, the present invention refers to the use of an RXR agonist in the manufacture of a pharmaceutical composition for treating a host having NIDDM, wherein said RXR agonist is LG 100268 or LGD 1069.

In a fourth aspect, the present invention refers to an in vitro method for increasing glucose uptake in adipose or muscle tissue, comprising the step of administering to said tissue a composition comprising an RXR agonist, said composition further comprising a peroxisome proliferator-activated receptor γ (PPARγ) agonist, said PPARγ agonist being a thiazolidinedione compound, wherein said RXR agonist is LG 100268 or LGD 1069, and wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120, 744, englitazone, AD 5075 and darglitazone.

In still another aspect, the present invention refers to an in vitro method for increasing glucose uptake in adipose or muscle tissue, comprising the step of administering to said tissue a composition comprising an RXR agonist, wherein said RXR agonist is LG 100268 or LGD 1069.

The compositions of this invention are adapted to cure, improve or prevent one or more symptoms of NIDDM in the host. A preferred drug is highly potent and selective with low toxicity. In this regard, those skilled in the art will recognize NIDDM as an example of a metabolic disease that can be treated with the RXR agonist-containing compounds and compositions of the present invention. Other examples of metabolic diseases treatable with the compounds and compositions of the present invention include obesity and thyroid hormone abnormalties.

**[0007]** By "pharmaceutically effective amount" is meant an amount of a pharmaceutical compound or composition having a therapeutically relevant effect on NIDDM. A therapeutically relevant effect relieves to some extent one or more symptoms of NIDDM in a patient or returns to normal either partially or completely one or more physiological or biochemical parameters associated with or causative of NIDDM, e.g. increasing the sensitivity of cellular response to circulating insulin, curing, reducing, or preventing one or more clinical symptoms of NIDDM, including, but not limited to, hyperglycemia, hyperinsulinemia and hypertriglyceridemia. In a preferred embodiment, a pharmaceutically effective amount of a compound or composition means an amount that increases the uptake of glucose by adipose tissue or muscle tissue. In another preferred embodiment, a pharmaceutically effective amount of a compound or composition means an amount that increases the uptake of triglyceride by adipose tissue.

**[0008]** By "activator of the RXR/PPARγ heterodimer" is meant a compound or composition which when combined with the RXR/PPARγ heterodimer increases the transcriptional regulation activity of the heterodimer, as measured by an assay known to one skilled in the art, including the "co-transfection" or "cis-trans" assays described or disclosed in U.S. Patent Nos. 4,981,784, 5,071,773, 5,298,429, 5,506,102, WO89/05355, WO91/06677, WO92/05447, WO93/11235, WO95/18380, PCT/US93/04399, PCT/US94/03795 and CA 2,034,220. It includes compounds that bind RXR, PPARγ, or both.

**[0009]** By "RXR agonist" is meant a compound or composition which when combined with RXR homodimers or heterodimers increases the transcriptional regulation activity of RXR, as measured by an assay known to one skilled in the art, including the "co-transfection" or "cis-trans" assays described or disclosed in U.S. Patent Nos. 4,981,784, 5,071,773, 5,298,429, 5,506,102, WO89/05355, WO91/06677, WO92/05447, WO93/11235, WO95/18380, PCT/US93/04399, PCT/US94/03795 and CA 2,034,220. It includes compounds that preferentially activate RXR over RAR (i.e. RXR specific agonists), and compounds that activate both RXR and RAR (i.e. pan agonists). It also includes compounds that activate RXR in a certain cellular context but not others (i.e. partial agonists). RXR specific agonists used here are LG 100268 and LGD 1069 and pharmaceutically acceptable salts thereof. The structures and syntheses of LG 100268 and LGD 1069 are disclosed in Boehm, et al. J. Med. Chem. 38(16):3146-3155, 1994. Pan agonist is ALRT 1057 (i.e. 9-cis retinoic acid), and pharmaceutically acceptable salts thereof.

**[0010]** In the embodiment according to the invention, the pharmaceutical composition contains a pharmaceutically effective amount of a PPARγ agonist.

**[0011]** By "PPARγ agonist" is meant a compound or composition which when combined with PPARγ increases a reaction typical for the receptor, e.g., transcriptional regulation activity, as measured by an assay known to one skilled in the art, including the "co-transfection" or "cis-trans" assays described or disclosed in U.S. Patent Nos. 4,981,784 and 5,071,773 and Lehmann, et al., J. Biol. Chem. 270:12953-12956 (1995). The PPARγ agonist used here is a thiazolidinedione compound, including BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120,744, englitazone, AD 5075, darglitazone and pharmaceutically acceptable salts thereof.

**[0012]** In a further preferred embodiment, the pharmaceutical composition also contains a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer, or insulin mimetic. Alternatively, a composition containing a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer, or insulin mimetic is administered to the host separately.

**[0013]** A composition containing a pharmaceutically effective amount of an active ingredient may be administered orally or systemically to a host. In a preferred embodiment, it is administered orally.

**[0014]** In another aspect, this invention features a pharmaceutical composition for treating NIDDM containing a pharmaceutically effective amount of the RXR agonist LG 100268 or LGS 1069; and a pharmaceutically acceptable carrier adapted for a host having NIDDM. In a preferred embodiment, the pharmaceutical composition also includes a pharmaceutically effective amount of insulin, insulin derivative, insulin secretagogue, insulin sensitizer, insulin mimetic or PPARγ agonist being a thiazolidinedione.

**[0015]** In a preferred embodiment, the composition is held within a container which includes a label stating to the effect that the composition is approved by the FDA in the United States (or an equivalent regulatory agency in a foreign country) for treating NIDDM or for treating hyperglycemia, hyperinsulinemia or hypertriglyceridemia. Such a container provides a therapeutically effective amount of the active ingredient to be administered to a host.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0016]**

Figure 1a is a graph showing the extent of adipocyte differentiation in 3T3-LI cells as measured by the levels of triglyceride in 3T3-LI preadipocytes treated with various combinations of an RXR agonist (LG 100268), a PPARγ agonist (thiazolidinedione compound BRL 49653), and insulin. The retinoid and BRL 49653 were-used at 1 μM.

Figure 1b is a graph showing the extent of adipocyte differentiation in 3T3-LI cells as measured by the level of triglyceride in 3T3-LI preadipocytes treated with various combinations of an RXR agonist (LGD 1069), a PPARγ agonist (thiazolidinedione compound BRL 49653), and insulin. The retinoid and BRL 49653 were used at 1 μM.

Figure 2a is a graph showing the level of LPL mRNA in 3T3-LI cells treated with various combinations of an RXR agonist (LG 100268), a PPARγ agonist (thiazolidinedione compound BRL 49653), and insulin. The retinoid and BRL 49653 were used at 1 μM.

Figure 2b is a graph showing the level of PPARγ mRNA in 3T3-LI cells treated with various combinations of an RXR agonist (LG 100268), a PPARγ agonist (thiazolidinedione compound BRL 49653), and insulin. The retinoid and BRL 49653 were used at 1 μM.

Figure 3 is a graph showing the level of glucose in *db/db* mice treated with LG 100268, BRL 49653, and a combination of LG 100268 and BRL 49653, respectively.

Figure 4 is a graph showing the level of triglycerides in *db/db* mice treated with LG 100268, BRL 49653, and a combination of LG 100268 and BRL 49653, respectively.

Figure 5 is a graph showing the level of HDL cholesterol in *db/db* mice treated with LG 100268, BRL 49653, and a combination of LG 100268 and BRL 49653, respectively.

Figure 6 is a graph showing the level of triglycerides in *ob/ob* mice treated with LGD 1069, LG 100268, and BRL 49653, respectively.

Figure 7 is a graph showing the level of glucose in ob/ob mice treated with LGD 1069, LG 100268, and BRL 49653, respectively.

Figure 8 is a graph showing the level of insulin in ob/ob mice treated with LGD 1069, LG 100268, and BRL 49653, respectively.

## DETAILED DESCRIPTION OF THE INVENTION

### TZD achieves antidiabetic and adipogenic effects through PPARγ

**[0017]** Thiazolidinediones are insulin sensitizers that significantly reduce glucose and lipid levels in animal models of NIDDM and obesity (Kees, et al., J. Medicinal Chem. 38(4):617-628, 1995; Willson, et al., J. Medicinal Chem. 39 (3):665-668, 1996; Young, et al. Diabetes 44:1087-1092, 1995). Thiazolidinediones improve glucose utilization without stimulating insulin release.

**[0018]** For example, repeated administration of BRL 49653 to obese mice improves glycemic control by increasing insulin responsiveness of target tissues. BRL 49653 potentiates insulin-stimulated glucose transport in adipocytes from insulin-resistant obese mice, both by increasing insulin receptor number and by facilitating translocation of GLUT4, from an expanded intracellular pool, to the cell surface.

**[0019]** Thiazolidinediones are also selective PPARγ agonists (Lehmann, et al. J. Biol. Chem. 270(22):1-4, 1995). Comparison of the $EC_{50}$ for activation of PPARγ with the minimum effective dose (MED) for anti-hyperglycemic activity revealed a significant correlation. The correlation between *in vitro* PPARγ activity and *in vivo* antihyperglycemic activity of thiazolidinediones implicates PPARγ as the molecular target for the antidiabetic effects of thiazolidinediones.

**[0020]** PPARγ is a member of the nuclear receptor superfamily of ligand-activated transcription factors. It is expressed in an adipose-specific manner and its expression is induced early during the differentiation of several preadipocyte cell lines. Forced expression of PPARγ in fibroblasts resulted in adipocyte differentiation.

**[0021]** In addition to insulin sensitizing activity, thiazolidinediones have marked adipogenic effects on preadipocyte and mesenchymal stem cells (Tontonoz, et al., Cell 79:1147-1156, 1994). Treatment of C3H/10T1/2 cells with BRL 49653 resulted in efficient adipocyte differentiation, showing that ligand-mediated activation of PPARγ is sufficient to initiate the adipogenic signaling cascade in a mesenchymal stem cell line. PPARγ is the molecular target for the adipogenic effects of thiazolidinediones.

**[0022]** Adipogenesis plays a role in the development of NIDDM, which is characterized by not only unbalanced glucose homeostasis, but also elevated levels of circulating lipids. Increases in lipid levels have been shown to interfere with glucose disposal.

**[0023]** Adipocytes are highly specialized cells that play a critical role in lipid metabolism and energy homeostasis. Their primary role is to store triglycerides in times of caloric excess and to mobilize this reserve during periods of

nutritional deprivation.

**[0024]** Adipocyte differentiation is characterized by a coordinate increase in adipocyte-specific gene expression. PPARγ is specifically expressed in adipocytes. Its expression is induced early during the course of differentiation of several preadipocyte cell lines. Forced expression of PPARγ in fibroblasts resulted in adipocyte differentiation.

Synergistic effects of RXR and PPARγ on adipogenesis

**[0025]** Expression of PPARγ is induced early during the differentiation of cultured adipocyte cell lines and is expressed at very high levels specifically in adipose tissue. PPARγ regulates adipogenesis by modulating the transcription of other adipocyte-specific genes, e.g. adipocyte P2 gene (aP2 gene). aP2 gene encodes an intracellular lipid-binding protein and is expressed exclusively in adipose cells.

**[0026]** A 518-bp DNA fragment from the 5'-flanking region of the aP2 gene has been identified as an enhancer that directs high-level adipocyte-specific gene expression in both cultured cells and transgenic mice. A pair of elements in the aP2 enhancer, ARE6 and ARE7, bind a nuclear factor termed ARF6 that is detected only in nuclear extracts derived from adipocytes. The ARF6-binding sites are both necessary and sufficient for adipocyte-specific expression, suggesting that the trans-acting factor ARF6 functions as a differentiation-dependent and tissue-specific switch for the aP2 enhancer (Tontonoz, et al. Genes & Development, 8:1224-1234, 1994).

**[0027]** The ARF6 recognition sequence resembles a type of nuclear hormone receptor-binding site known as DR-1 (direct repeat with 1-nucleotide spacer). This motif has been shown to preferentially bind heterodimers of RXR and COUP-TF and heterodimers of RXR and the PPARs. DNA mobility retardation experiments using various HRE sequences as competitor demonstrated that ARF6 preferentially recognizes DR-1 sites.

**[0028]** ARF6 has been identified as a heterodimeric complex of RXRα and PPARγ. It has been shown that PPARγ and RXRα form heterodimers on ARF6-binding sites *in vitro*. Forced expression of these factors in transient transfections is sufficient to activate the adipocyte-specific aP2 enhancer in nonadipose cells such as fibroblasts. This activation is potentiated by peroxisome proliferators, fatty acids, and 9-*cis* retinoic acid. Antiserum to RXRα specifically inhibits ARF6 activity in adipocyte nuclear extracts.

**[0029]** Cotransfection of the RXRα expression vector and the PPARγ expression vector has a synergistic effect to activate the aP2 enhancer in nonadipose cells. Maximal activation of the aP2 enhancer is observed when both PPARγ, RXRα and their agonists are present.

**[0030]** Without being bound by any theory, applicant proposes that an RXR agonist affects glucose usage in tissues through the synergistic effects of RXR and PPARγ heterodimers. The RXR/PPARγ heterodimers, when activated by an RXR agonist or a combination of an RXR agonist and a PPARγ agonist, induce adipogenesis and modulates the levels of glucose and triglyceride uptake. Alternatively or in addition, the RXR/PPARγ heterodimers, when activated by an RXR agonist or a combination of an RXR agonist and a PPARγ agonist, regulate signaling molecules secreted by adipose tissue such as tumor necrosis factor-α or leptin, which in turn modulates glucose metabolism in other tissues.

Using RXR agonists to mimic or enhance the antidiabetic effects of thiazolidinediones

**[0031]** PPARα, β and γ all form heterodimers with RXRs. These RXR/PPAR heterodimers bind to DNA and regulate transcription activity. RXR activators cooperate with PPARα activators to activate the activity of PPARα protein (Kliewer, *et al.* Nature 358:771-774 (1992) and Mukherjee, *et al.* Steroid Biochem. Molec. Biol. 51:157-166 (1994)).

**[0032]** In this invention, a similar synergistic activation was observed with a PPARγ activator and several RXR activators.

**[0033]** According to this invention, the RXR agonists LGD 1069, ALRT 1057 and LG 100268, may be utilized in the treatment of diabetes. We examined four independent parameters for the effects of RXR agonists, i.e. morphological changes, lipid accumulation, regulation of gene expression and increased glucose uptake.

**[0034]** Two pre-adipocyte cell lines were used to test the theory of RXR activation in the PPARγ/RXR heterodimer. 3T3-LI and C3H/10T1/2 cells were obtained from ATCC and are derived from mouse embryo. They are contact inhibited and can be induced to differentiate into adipocyte cells containing large lipid droplets within the cytoplasm. Adipocyte differentiation can be observed by oil red O staining which stains the lipid droplets within the cytoplasm red. The extent of adipocyte differentiation can then be monitored by microscope observation.

**[0035]** For a more quantitative assay and rapid screening of compounds, a 96-well plate assay was developed to quantitate the amount of triglyceride produced by the differentiating adipocytes. In this assay, cells are grown as a monolayer to confluence on a 96-well plate and treated with BRL 49653, insulin, and retinoids alone or in various combinations. These treatments induce differentiation to different extent in both 3T3-LI and C3H/10T1/2 cells. The level of triglyceride accumulation can then be measured via an enzymatic color reaction which can be read in a plate reader.

**[0036]** A third measure of adipocyte differentiation is to examine regulation of gene expression. The mRNA expres-

sion levels of both PPARγ and lipoprotein lipase (LPL) have been shown to be modulated during adipocyte differentiation. Northern blot analysis was used to dissect the molecular aspects of how retinoids effect target genes of differentiating adipocytes. PPARγ, lipoprotein lipase (LPL), and β-actin (loading control) mRNA levels were monitored after cells were treated with thiazolidinediones and retinoids.

**[0037]** A fourth indicator for the utility of a compound in treating NIDDM or insulin-resistant diabetes is the compound's ability to enhance insulin-stimulated glucose uptake. Labeled 2-deoxyglucose (2-DOG, a glucose analog) assay was performed with a preadipocyte cell line in the presence of insulin and a candidate compound to measure the level of 2-DOG incorporation.

A) Retinoid Modulation of Lipid Accumulation in 3T3-LI Cells Stained with Oil Red O

**[0038]** Table 1 shows the percent of 3T3-LI cells that had differentiated into adipocytes as observed by oil red O staining assay. BRL 49653 and LG 100268 were used at 1 μM, insulin was used at 0.01 mg/ml. Wells treated with LG 100268 had bigger redder lipid droplets within the cytoplasm.

**[0039]** BRL 49653 and LG 100268 treatment alone induced 50% of the cells to differentiate into adipocytes. This was increased dramatically with the addition of insulin. Insulin in combination with BRL induced 80% of the 3T3-LI cells into adipocytes while the combination of insulin and LG 100268 induced 90% of the cells to differentiate. When BRL 49653 was used in combination with LG 100268, an RXR agonist, the amount of adipocytes differentiation was also increased dramatically. Other RXR agonists mimic the activity of LG 100268. For example, the addition of ALRT 1057 (pan agonist) or LGD 1069 (RXR specific agonist) in combination with BRL 49653 increased the amount of differentiation, albeit to a lesser extent than the strong RXR agonist LG 100268. The combination of insulin with BRL 49653 and LGD 1069 had a strong differentiating effect (95%) on the 3T3-LI cells.

B) Retinoid Modulation of Triglyceride Content in Differentiated Adipocytes

**[0040]** The retinoid modulation of lipid formation was quantitated by monitoring triglyceride formation. Figures 1a and 1b show triglyceride accumulation in 3T3-LI cells treated with a retinoid (LG 100268 or LGD 1069) alone or in combination with a thiazolidinedione and insulin. Retinoids and BRL 49653 were used at 1 μM, insulin was used at 0.01 mg/ml for all experimental combinations.

**[0041]** Insulin, BRL 49653 and retinoids all induced some triglyceride accumulation when used alone, with LG 100268 giving the largest response. The addition of retinoids (LGD 1069, LG 100268) with the thiazolidinedione (BRL 49653) to the assay increased the amount of triglyceride accumulation in 3T3-LI differentiating adipocytes. This was also observed when BRL 49653 or LG 100268 was used in combination with insulin. The largest accumulation of triglyceride was seen in the cells treated with LG 100268, BRL 49653 and insulin together. Similar results were observed when LGD 1069 replaced LG 100268 in the study. These results concur with those obtained in the oil red O staining assays.

C) PPARγ and LPL mRNA modulation in differentiating 3T3-LI cells

**[0042]** Adipocyte specific genes were monitored via northern blot analysis. Figure 2a and 2b show the expression pattern of LPL (lipoprotein lipase) mRNA and PPARγ mRNA in cells that were treated for 7 days with BRL 49653 (1 μM), LG 100268 (1 μM) and insulin (0.01 mg/ml), alone or in combination.

**[0043]** Northern blot analysis shows an increase in the relative signal normalized to β-actin of both LPL and PPARγ mRNA expression in cells treated with either compound alone. There was a three to five fold increase in mRNA levels for these adipocyte target genes demonstrating that transcriptional regulation occurs with treatment by insulin, BRL 49653 and LG 100268. Combination of insulin, BRL 49653 and LG 100268 did not further enhance the mRNA level.

**[0044]** These data demonstrate that, in 3T3-LI cells, RXR agonists induce adipocyte differentiation by themselves or in combination with thiazolidinediones or insulin. RXR agonists enhance the activity of thiazolidinediones and insulin. Three independent measurements support that RXR agonists contribute to the modulation of the RXR/PPARγ heterodimer in regulating adipocyte differentiation and useful in treating NIDDM.

D) LG 100268 enhances insulin stimulated glucose uptake in 3T3-LI cells

**[0045]** A murine preadipocyte cell line, 3T3-L1, is widely used to study glucose uptake, adipogenesis, and has been used in the characterization of thiazolidinediones and other PPARγ activators. Insulin stimulated uptake of labeled 2-deoxyglucose (2-DOG, a glucose analog) was observed in 3T3-L1 cells treated with BRL 49653 or LG 100268.

**[0046]** 3T3-L1 cells were treated with BRL 49653 (10 μM) or LG 100268 (1 μM) for 10 days. Insulin was added to the cells at a concentration of 0.01 mg/ml for 5 days, thereafter no insulin was added. Labeled 2-deoxyglucose uptake assay was performed (Szalkowski, et al., J. Endocrin. 136:1474-1481, 1995). The level of incorporated labeled 2-DOG

was normalized to the amount of total cellular protein. About 5-fold increase in 2-DOG uptake was observed in 3T3-L1 cells treated with BRL 49653 alone. About 2-fold increase in 2-DOG uptake was observed in 3T3-L1 cells treated with LG 100268 alone.

**[0047]** This experiment shows that an RXR agonist increases insulin mediated glucose uptake in x3T3-L1 cells like a known insulin sensitizer, a thiazolidinedione. It demonstrates directly that RXR agonists can be used to treat a major symptom of NIDDM, *i.e.* insulin resistance. Other RXR agonists useful for the treatment of NIDDM can be confirmed using this assay.

E) Retinoid Modulation of Lipid Accumulation in C3H/10T1/2 Cells Stained with Oil Red O

**[0048]** To further assess the retinoid regulation of adipocyte function we examined C3H/10T1/2 cells, which are mouse embryo fibroblast/multipotent stem cells that can be induced to differentiate into adipocytes or muscle cells. Table 2 shows the percent of C3H/10T1/2 cells that have differentiated into adipocytes as observed by oil red O staining assay. Experiment A was conducted in the presence of BRL 49653. Experiment B was conducted in the presence of both BRL 49653 and insulin. ALRT 1057 and LGD 1069 were used at 1 μM and TTNPB (RAR selective compound) was used at 10 nM concentration. Insulin was used at 0.01 mg/ml; and BRL 49653 was used at 0.1, 1 and 10 μM. C3H/10T1/2 cells were treated for 7 days, cells were stained with oil red O and observed under the microscope.

**[0049]** Morphological changes were observed when the cells were treated with retinoids alone, even though the cells did not fully differentiate into adipocytes. BRL 49653 alone caused no more than 10 percent of the cells to undergo adipocyte differentiation. However, when BRL 49653 was used in combination with an RXR agonist, LGD 1069 or ALRT 1057, there was a large increase in the amount of differentiation, the same effect was seen when BRL 49653 was used in combination with insulin.

**[0050]** The RAR agonist, TTNPB (which does not activate RXR), did not have the same effect as RXR agonist LGD 1069 and ALRT 1057. In fact, TTNPB inhibited the differentiation induced by BRL 49653 or BRL/insulin.

**[0051]** The above experiments show that a thiazolidinedione (BRL 49653) alone induces a minimal amount of differentiation in C3H/10T1/2 cells. However, when BRL 49653 is used in combination with a retinoid such as LG 100268, LGD 1069, or ALRT 1057, which are RXR agonists, differentiation is dramatically increased. This is not seen with the pure RAR agonist, TTNPB. These data support that the PPARγ/RXR heterodimers, which drive the adipocyte differentiation process, can be activated and enhanced by the binding of an RXR agonist. RXR agonists are useful for modulating the levels of glucose and triglyceride uptake.

Using RXR agonists to lower levels of glucose and triglycerides in animal models of NIDDM

(A) *In vivo* experiment with *db/db* mice

**[0052]**

Animals: Strain Diabetic C57BLKS/J - *m +/+db*, 82 mice
Source: Jackson Lab
Stock number 000642
Genotype *m +/+db xm +/+db*
DOB 6/19/96±3d, DOA 7/23/96 - 34d old
Date of study: 8/5/96 - 8/21/96, 44-63d old

**[0053]** Mice were identified by an ear punch code (#1-82) and separated into 8 groups (A-H). Each group consisted of 10 mice separated into 4 cages with 2-3 mice/cage. Several mice were lost during the course of the study from fluid injections into the lungs resulting in 2 groups with 9 mice/group. Control group C consisted of 12 mice. The mice were fed pelleted Purina Lab chow #5015 containing 3.83kcal/g with a caloric composition of 56% carbohydrate, 26% fat and 18% protein. Food and water were provided ad libitum. Food intake/cage was measured over selected periods and expressed as g food consumed/100g mouse/day.

**[0054]** On days of study, food was removed from the cages at selected intervals of time between 6:15 and 7:00 AM. Animal weights were recorded 2h after start of fast and blood samples taken after a 3h fast. Blood was drawn from a cut at the tip of the tail and collected into a heparinized capillary tube (approx. 75μl volume). After centrifugation, the hematocrit was read in a microcapillary reader, recorded and the tube broken for recovery of plasma for analysis of glucose, triglyceride and insulin concentration.

**[0055]** Blood samples were collected on days $0_{-1}$, 0, 3, 7, 10, and on the final day of study, days 13-15. After collection of blood samples on day 0, animals were refed their Purina chow diet and subsequently gavaged with control solution in group C and one of seven test solutions in groups designated A, B, D-H. The volume administered was equivalent

in each group averaging .6ml/42g mouse (.01429 ml/g). The various solutions were gavaged daily to their respective groups based on the animals weight taken that morning or the weight of the animal taken on the preceding day of weighing. To assess alterations in plasma FFA, on day 10 an addition 75 µl blood sample was collected into an EDTA-coated capillary tube immediately after collecting the basal heparinized capillary tube sample.

**[0056]** On the final day of study, mice were not gavaged with test solution. The last gavage was administered on the day preceding the final day, i.e., day 12 for animals terminated on day 13, day 13 for animals terminated on day 14 and day 14 for animals terminated on day 15. Terminal blood was collected by decapitation to provide serum for assessment of HDL.

Results:

**[0057]** Figure 3 shows that BRL49653 and LG100268 each independently lowered the level of glucose in *db/db* mice. In addition, the combination of BRL49653 and LG100268 lowered the glucose level more than each compound did by itself. BRL 49653 (1 mg/kg) and LG 100268 (20 mg/kg) lowered the level of glucose by 40% by day 15 in comparison to control. The thiazolidinedione BRL 49653 at 1 mg/kg showed similar efficacy. The combination of an RXR activator and a PPAR activator showed greater efficacy, leading to almost 50% drop in the level of glucose. The effect of the combination was rapid, glucose levels were reduced by day 1 of the study and reached a steady state by day 4. This indicates a rapid resetting of the steady state levels of glucose homeostasis. Therefore, RXR activators enhance the efficacy of PPARγ activators, and vice vesa.

**[0058]** Figure 4 shows that RXR activators lowered the level of triglycerides in db/db mice. LG 100268 (20 mg/kg) lowered the level of triglycerides 40% by day 15 of the study. BRL 49653 (1 mg/kg) showed similar efficacy. The combination of these two compounds worked even better.

**[0059]** Figure 5 shows that RXR activators modulators increased the level of HDL cholesterol in db/db mice. LG 100268 (20 mg/kg) increased HDL-cholesterol levels (20%) in comparison to controls in db/db mice. BRL 49653 caused an equivalent level of increase. The combination of these two compounds showed a higher increase. HDL-C was measured by the precipitation method using kits obtained from Bohringer-Mannheim (catalog # 543004 and 427578).

(B) *In vivo* experiment with *ob/ob* mice

**[0060]**

Animals: Strain Obese C57 BL/6J-$Lep^{ab(4)}$, 121 mice
Source: Jackson Lab Stock number 000632
Genotype $Lep^{ob(4)}/+$ x $Lep^{ob(4)}/+$
DOB 5/22/96±3d, DOA 7/2/96 - 41d old
Date of study: 7/21/96 - 8/2/96 - 49-70d old

**[0061]** Mice were identified by an ear punch code (#1-121) and separated into 12 groups. Each group consisting of 10 mice. (one mouse was terminated before start of the study because of bad teeth causing initial weight loss. As in preceding studies, mice in each group were housed in 4 cages (2-3 mice/cage) and provided water and Purina Lab chow #505\15 *ad libitum*.

**[0062]** On days of study, food was removed from the cages at selected intervals of time between 6:15 and 7:00 AM. Animal weights were recorded 2h after start of fast and blood samples taken after a 3h fast. Blood was drawn from a cut at the tip of the tail and collected into a heparinized capillary tube (approx. 75µl volume). After centrifugation, the hematocrit was read in a microcapillary reader, recorded and the tube broken for recovery of plasma for analysis of glucose, triglyceride and insulin concentration.

**[0063]** Blood samples were collected on days $0_{-3}$, 0, 3, 6, 8, 10, 14±1 and final collections were made on days 15, 16 or 17. FFA samples were collected on day 10 by drawing a second 75µl blood sample in a non-heparinized tube coated with EDTA. This tube was collected immediately after the heparinized tube collection.

**[0064]** Animals in groups H were administered control gavage solution (0.6 ml/42 g) daily commencing on day 0 and ending on the day preceding the final day. 11 test solutions were administered to animals in groups designated A-G, I-L. All gavage solutions were administered after refeeding on days in which mice were fasted for blood sampling.

Results:

**[0065]** Figure 6 shows that RXR modulators lowered the level of triglycerides in *ob/ob* mice. RXR activators LGD 1069 (30 mg/kg) and LG 100268 (20 mg/kg) lowered the level of triglycerides by 34% and 60% respectively in *ob/ob* mice by day 14 of the study. BRL 49653 was also able to lower the level of triglycerides, although not as efficacious

as LG100268.

**[0066]** Figure 7 shows that RXR modulators lowered the level of glucose in *ob/ob* mice. RXR activators LGD 1069 at 30 mg/kg and LG 100268 at 20 mg/kg lowered the level of glucose nearly 50% in comparison to control. The level of glucose was reduced to almost euglycemic levels by day 14 of the study. BRL 49653 (0.4 mg/kg) showed similar efficacy.

**[0067]** Figure 8 shows that RXR modulators, LGD 1069 (30 mg/kg) and LG 100268 (20 mg/kg), lowered the level of insulin in *ob/ob* mice. LG100268 lowered the level of insulin by 66% by day 14 of the study. LGD 1069 showed lower efficacy. There was a very rapid effect of the compounds since the level of insulin started to drop in day 1.

Pharmaceutical Formulations and Modes of Administration

**[0068]** The particular compound that affects the disorders or conditions of interest can be administered to a patient either by themselves, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s). In treating a patient exhibiting a disorder of interest, a therapeutically effective amount of a agent or agents such as these is administered. A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient.

**[0069]** The compounds also can be prepared as pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts include acid addition salts such as those containing hydrochloride, sulfate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, cyclohexylsulfamate and quinate. (*See e.g.*, PCT/US92/03736). Such salts can be derived using acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, *p*-toluenesulfonic acid, cyclohexylsulfamic acid, and quinic acid.. These salts can be prepared by standard techniques. For example, the free base form of the compound is first dissolved in a suitable solvent such as an aqueous or aqueous-alcohol solution, containing the appropriate acid. The salt is then isolated by evaporating the solution. In another example, the salt is prepared by reacting the free base and acid in an organic solvent.

**[0070]** Carriers or excipients can be used to facilitate administration of the compound, for example, to increase the solubility of the compound. Examples of carriers and excipients include calcium carbonate, calcium phosphate, various sugars or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents.

**[0071]** In addition, the molecules tested can be used to determine the structural features that enable them to act on the RXR/PPARγ heterodimer, and thus to select useful molecules.

**[0072]** Those skilled in the art will know how to design drugs from lead molecules, using techniques such as those disclosed in PCT publication WO 94/18959.

**[0073]** Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the $LD_{50}$ (the dose lethal to 50% of the population) and the $ED_{50}$ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio $LD_{50}/ED_{50}$. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the $ED_{50}$ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. Levels in plasma may be measured, for example, by HPLC.

**[0074]** The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g. Fingl et al., in The Pharmacological Basis of Therapeutics, 1975, Ch. 1 p. 1). It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administrated dose in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

**[0075]** Depending on the specific conditions being treated, such agents may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA (1990). Suitable routes may include oral, rectal, transdermal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections,

just to name a few.

**[0076]** For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

**[0077]** Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

**[0078]** Agents intended to be administered intracellularly may be administered using techniques well known to those of ordinary skill in the art. For example, such agents may be encapsulated into liposomes, then administered as described above. Liposomes are spherical lipid bilayers with aqueous interiors. All molecules present in an aqueous solution at the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external microenvironment and, because liposomes fuse with cell membranes, are efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, small organic molecules may be directly administered intracellularly.

**[0079]** Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions. The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

**[0080]** Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

**[0081]** Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

**[0082]** Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

**[0083]** Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycals. In addition, stabilizers may be added. Liposomes may be used for encapsulated delivery.

**[0084]** Pharmaceutical formulations are disclosed or described in Boehm, et al., WO94/15902.

**[0085]** Embodiments of this invention are disclosed in the following claims.

TABLE 1:

| Oil Red O Staining in 3T3-L1 Differentiating Adipocytes | |
|---|---|
| Treatment | Percent of Differentiated Cells |
| Control | 0.1 |
| BRL 49653 | 50 |
| Insulin | 20 |
| LG 100268 | 50 |
| BRL+Insulin | 80 |
| BRL+LG 100268 | 90 |
| LG 100268+Insulin | 90 |

TABLE 2:

| Retinoid Modulation of Adipocyte differentiation in C3H/10T1/2 Cells | | |
|---|---|---|
| Treatment | Experiment A (+BRL) % differentiated cells | Experiment B (+BRL+Insulin) % differentiated cells |
| BRL (10μM) | 10 | 80 |
| BRL (1μM) | 8 | 80 |
| BRL (0.1μM) | 5 | 60 |
| BRL (10μM) | 10 | 80 |
| +ALRT 1057 (1μM) | 80 | 99 |
| +LGD 1069(1μM) | 80 | 99 |
| +TTNPB(10nM) | 5 | 50 |
| BRL (1μM) | 8 | 80 |
| +ALRT 1057 (1μM) | 65 | 90 |
| +LGD 1069(1μM) | 65 | 90 |
| +TTNPB(10nM) | 2 | 30 |
| BRL (0.1μM) | 5 | 60 |
| +ALRT 1057(1μM) | 45 | 85 |
| +LGD 1069(1μM) | 45 | 90 |
| +TTNPB (10nM) | 0.2 | |
| Undifferentiated controls 0% differentiation. | | |

**Claims**

1. Use of an Retinoid X Receptor (RXR) agonist in the manufacture of a pharmaceutical composition for treating non-insulin-dependent diabetes mellitus (NIDDM) wherein said composition further comprises a peroxisome proliferator-activated receptor γ (PPARγ) agonist, said PPARγ agonist being a thiazolidinedione compound, wherein said RXR agonist is 2-[1-3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-cyclopropyl]-pyridine-5-carboxylic acid (LG 100268) or 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)ethenyl]benzoic acid (LGD 1069).

2. The use of claim 1, wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120,744, englitazone, AD 5075 and darglitazone.

3. The use of claim 1 or 2, wherein said composition further comprises insulin, insulin derivative, insulin secretagogue, insulin sensitizer or insulin mimetic.

4. A pharmaceutical composition for the treatment of NIDDM comprising

(a) an RXR agonist;
(b) a PPARγ agonist; and
(c) a pharmaceutically acceptable carrier,

wherein said PPARγ agonist is a thiazolidinedione compound, and wherein said RXR agonist is selected from the group consisting of LG 100268, LGD 1069 or 9-cis retinoic acid.

5. The composition of claim 4, wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitazone, ciglitazone, WAY-120,744, englitazone, AD 5075 and darglitazone.

6. The composition of claim 5 further comprising insulin, insulin derivative, insulin secretagogue, insulin sensitizer or insulin mimetic.

7. The use of an RXR agonist in the manufacture of a pharmaceutical composition for treating NIDDM, wherein said RXR agonist is LG 100268 or LGD 1069.

8. The use of claim 7, wherein said composition further comprises insulin, insulin derivative, insulin secretagogue.

9. In vitro method for increasing glucose uptake in adipose or muscle tissue, comprising the step of administering to said tissue a composition comprising an RXR agonist, said composition further comprising a peroxisome proliferator-activated receptor γ (PPARγ) agonist, said PPARγ agonist being a thiazolidinedione compound, wherein said RXR agonist is LG 100268 or LGD 1069, and wherein said thiazolidinedione compound is selected from the group consisting of BRL 49653, troglitazone, pioglitatzone, ciglitazone, WAY-120,744, englitazone, AD 5075 and darglitazone.

10. The method of claim 9, further comprising the step of administering to said tissue a composition comprising insulin, insulin derivative, insulin secretagogue, insulin sensitizer or insulin mimetic.

11. In vitro method for increasing glucose uptake in adipose or muscle tissue, comprising the step of administering to said tissue a composition comprising an RXR agonist, wherein said RXR agonist is LG 100268 or LGD 1069.

12. The method of claim 11, wherein said composition further comprises insulin, insulin derivative, insulin secretagogue.

**Patentansprüche**

1. Verwendung eines Retinoid-X-Rezeptor(RXR)-Agonisten für die Herstellung einer pharmazeutischen Zusammensetzung zur Behandlung von nicht-insulinabhängigem Diabetes mellitus (NIDDM), wobei die Zusammensetzung ferner einen Peroxysom-Proliferator-aktivierten Rezeptor-γ(PPARγ)-Agonisten umfasst, wobei der PPARγ-Agonist eine Thiazolidindionverbindung ist, wobei der RXR-Agonist 2-[1-3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-cyclopropyl]-pyridin-5-carbonsäure (LG 100268) oder 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalinyl)ethenyl]benzoesäure (LGD 1069) ist.

2. Die Verwendung nach Anspruch 1, wobei die Thiazolidindionverbindung aus der Gruppe ausgewählt wird, die aus BRL 49653, Troglitazon, Pioglitazon, Ciglitazon, WAY-120,744, Englitazon, AD 5075 und Darglitazon besteht.

3. Die Verwendung nach Anspruch 1 oder 2, wobei die Zusammensetzung ferner Insulin, ein Insulinderivat, ein Insulinsekretagog, einen Insulin-Sensitizer oder ein Insulin-Mimetic umfasst.

4. Pharmazeutische Zusammensetzung zur Behandlung von NIDDM, die umfasst,

(a) einen RXR-Agonisten;
(b) ein PPARγ-Agonisten; und
(c) einen pharmazeutisch geeigneten Träger,

wobei der PPARγ-Agonist eine Thiazolidindionverbindung ist und wobei der RXR-Agonist aus der Gruppe ausgewählt wird, die aus LG 100268, LGD 1069 oder 9-cis-Retinolsäure besteht.

5. Die Zusammensetzung nach Anspruch 4, wobei die Thiazolidindionverbindung aus der Gruppe ausgewählt wird, die aus BRL 49653, Troglitazon, Pioglitazon, Ciglitazon, WAY-120,744, Englitazon, AD 5075 und Darglitazon besteht.

6. Die Zusammensetzung nach Anspruch 5, die ferner Insulin, ein Insulinderivat, Insulinsekretagog, Insulin-Sensitizer oder Insulin-Mimetic umfasst.

7. Die Verwendung eines RXR-Agonisten für die Herstellung einer pharmazeutischen Zusammensetzung zur Behandlung von NIDDM, wobei der RXR-Agonist LG 100268 oder LGD 1069 ist.

8. Die Verwendung nach Anspruch 7, wobei die Zusammensetzung ferner Insulin, Insulinderivat, Insulinsekretagog umfasst.

9. In vitro-Verfahren zur Steigerung der Glukoseaufnahme in Fett- oder Muskelgewebe, das den Schritt umfasst, eine Zusammensetzung, die einen RXR-Agonisten umfasst, wobei die Zusammensetzung ferner einen Peroxysom-Proliferator-aktivierten Rezeptor-γ(PPARγ)-Agonisten umfasst, wobei der PPARγ-Agonist eine Thiazolidindionverbindung ist, wobei der RXR-Agonist LG 100268 oder LGD 1069 ist und wobei die Thiazolidindionverbindung aus der Gruppe ausgewählt wird, die aus BRL 49653, Troglitazon, Pioglitazon, Ciglitazon, WAY-120,744, Englitazon, AD 5075 und Darglitazon besteht, dem Gewebe zu verabreichen.

10. Das Verfahren nach Anspruch 9, das ferner den Schritt umfasst, eine Zusammensetzung, die Insulin, Insulinderivat, Insulinsekretagog, Insulin-Sensitizer oder Insulin-Mimetic umfasst, dem Gewebe zu verabreichen.

11. In vitro-Verfahren zur Steigerung der Glukoseaufnahme in Fett- oder Muskelgewebe, das den Schritt umfasst, eine Zusammensetzung, die einen RXR-Agonisten umfasst, wobei der RXR-Agonist LG 100268 oder LGD 1069 ist, dem Gewebe zu verabreichen.

12. Das Verfahren nach Anspruch 11, wobei die Zusammensetzung ferner Insulin, Insulinderivat, Insulinsekretagog umfasst.

**Revendications**

1. Utilisation d'un agoniste de récepteur de rétinoïde X (RXR) pour la préparation d'une composition pharmaceutique destinée à traiter le diabète sucré non insulino-dépendant (NIDDM), dans laquelle ladite composition comprend en outre un agoniste de récepteur γ activé par agent de prolifération de peroxysome (PPARγ), ledite agoniste de PPARγ étant un composé de type thiazolidinedione, et dans laquelle ledit agoniste de RXR est l'acide 2-[1-(3,5,5,8,8-pentaméthyl-5,6,7,8-tétrahydro-2-naphtyl)-cyclopropyl]pyridine-5-carboxylique (LG 100268) ou l'acide 4-[1-(5,6,7,8-tétrahydro-3,5,5,8,8-pentaméthyl-2-naphalényl)éthényl]benzoïque (LGD 1069).

2. Utilisation selon la revendication 1, dans laquelle ledit composé de type de thiazolidinedione est choisi parmi le groupe consistant en le composé BRL 49653, la troglitazone, la pioglitazone, la ciglitazone, le composé WAY-120 744, l'englitazone, le composé AD 5075 et la darglitazone.

3. Utilisation selon la revendication 1 ou 2, dans laquelle ladite composition comprend en outre de l'insuline, un dérivé d'insuline, un sécrétagogue d'insuline, un agent sensibilisant à l'insuline ou un agent mimétique de l'insuline.

4. Composition pharmaceutique pour le traitement du diabète NIDDM, comprenant :

   (a) un agoniste de RXR ;
   (b) un agoniste de PPARγ ; et
   (c) un véhicule pharmaceutiquement acceptable,

   dans laquelle ledit agoniste de PPARγ est un composé dérivé de thiazolidinedione, et dans laquelle ledit agoniste de RXR est choisi parmi le groupe consistant en le composé LG 100268, le composé LGD 1069 ou l'acide 9-cis-rétinoïque.

5. Composition selon la revendication 4, dans laquelle ledit composé de type thiazolidinedione est choisi parmi le

groupe consistant en le composé BRL 49653, la troglitazone, la pioglitazone, la ciglitazone, le composé WAY-120 744, l'englitazone, le composé AD 5075 et la darglitazone.

6. Composition selon la revendication 5, comprenant en outre de l'insuline, un dérivé d'insuline, un sécrétagogue d'insuline, un agent sensibilisant à l'insuline ou un agent mimétique de l'insuline.

7. Utilisation d'un agoniste de RXR pour la préparation d'une composition pharmaceutique destinée à traiter le diabète NIDDM, dans laquelle ledit agoniste de RXR est le composé LG 100268 ou le composé LGD 1069.

8. Utilisation selon la revendication 7, dans laquelle ladite composition comprend en outre de l'insuline, un dérivé d'insuline, un sécrétagogue d'insuline.

9. Procédé in vitro d'accroissement de l'absorption du glucose dans un tissu adipeux ou musculaire, comprenant les étapes consistant à administrer dans ledit tissu, une composition comprenant un agoniste de RXR, ladite composition comprenant en outre un agoniste de récepteur γ activé par agent de prolifération de peroxysome (PPARγ), ledit agoniste de PPARγ étant un composé de type thiazolidinedione, dans lequel ledit agoniste de RXR est le composé LG 100268 ou le composé LGD 1069, et dans lequel ledit composé de type thiazolidinedione est choisi parmi le groupe consistant en le composé BRL 49653, la troglitazone, la pioglitazone, la ciglitazone, le composé WAY-120 744, l'englitazone, le composé AD 5075 et la darglitazone.

10. Procédé selon la revendication 9, comprenant en outre l'étape consistant à administrer dans ledit tissu, une composition comprenant de l'insuline, un dérivé d'insuline, un sécrétagogue d'insuline, un agent sensibilisant à l'insuline ou un agent mimétique de l'insuline.

11. Procédé in vitro d'accroissement de l'absorption du glucose dans un tissu adipeux ou musculaire, comprenant l'étape consistant à administrer dans ledit tissu, une composition comprenant un agoniste de RXR, ledit agoniste de RXR étant le composé LG 100268 ou le composé LGD 1069.

12. Procédé selon la revendication 11, dans lequel ladite composition comprend en outre de l'insuline, un dérivé d'insuline, un sécrétagogue d'insuline.

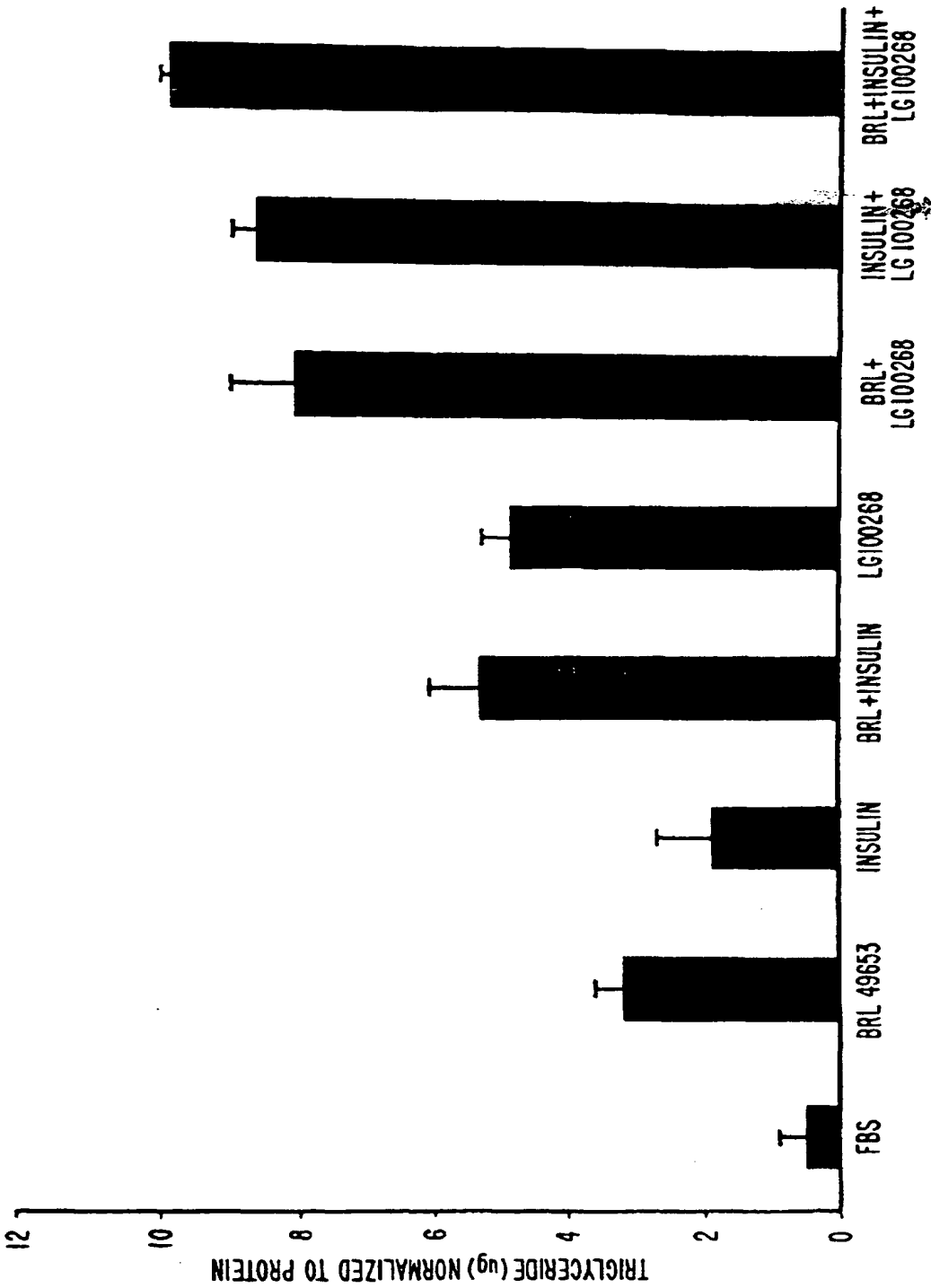

TRIGLYCERIDE (ug) NORMALIZED TO PROTEIN
0
2
4
6
8
10
12
FBS
BRL 49653
INSULIN
BRL+INSULIN
LG100268
BRL+ LG100268
INSULIN+ LG100268
BRL+INSULIN+ LG100268

FIG. 1a.

FIG. 1b.

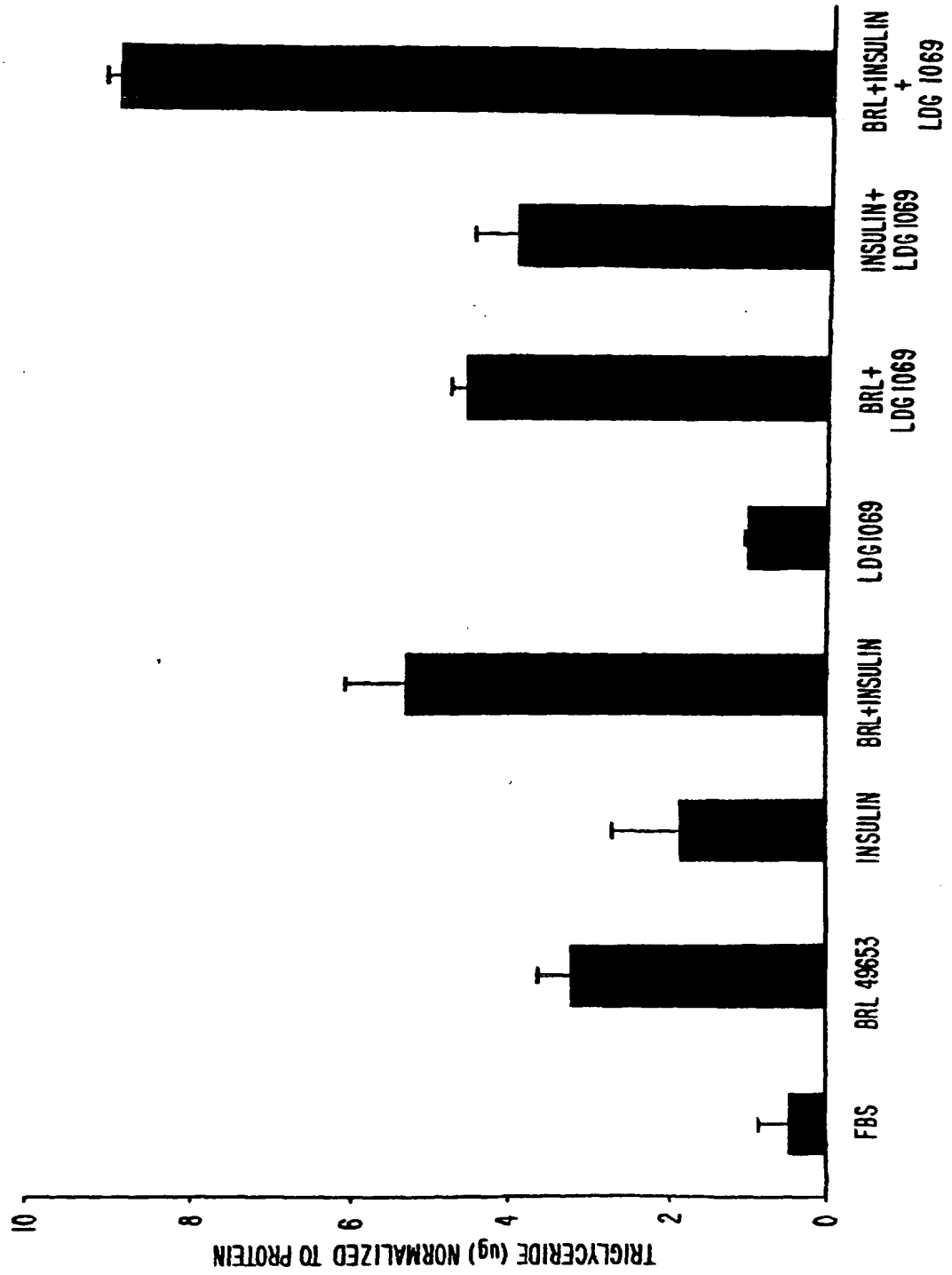
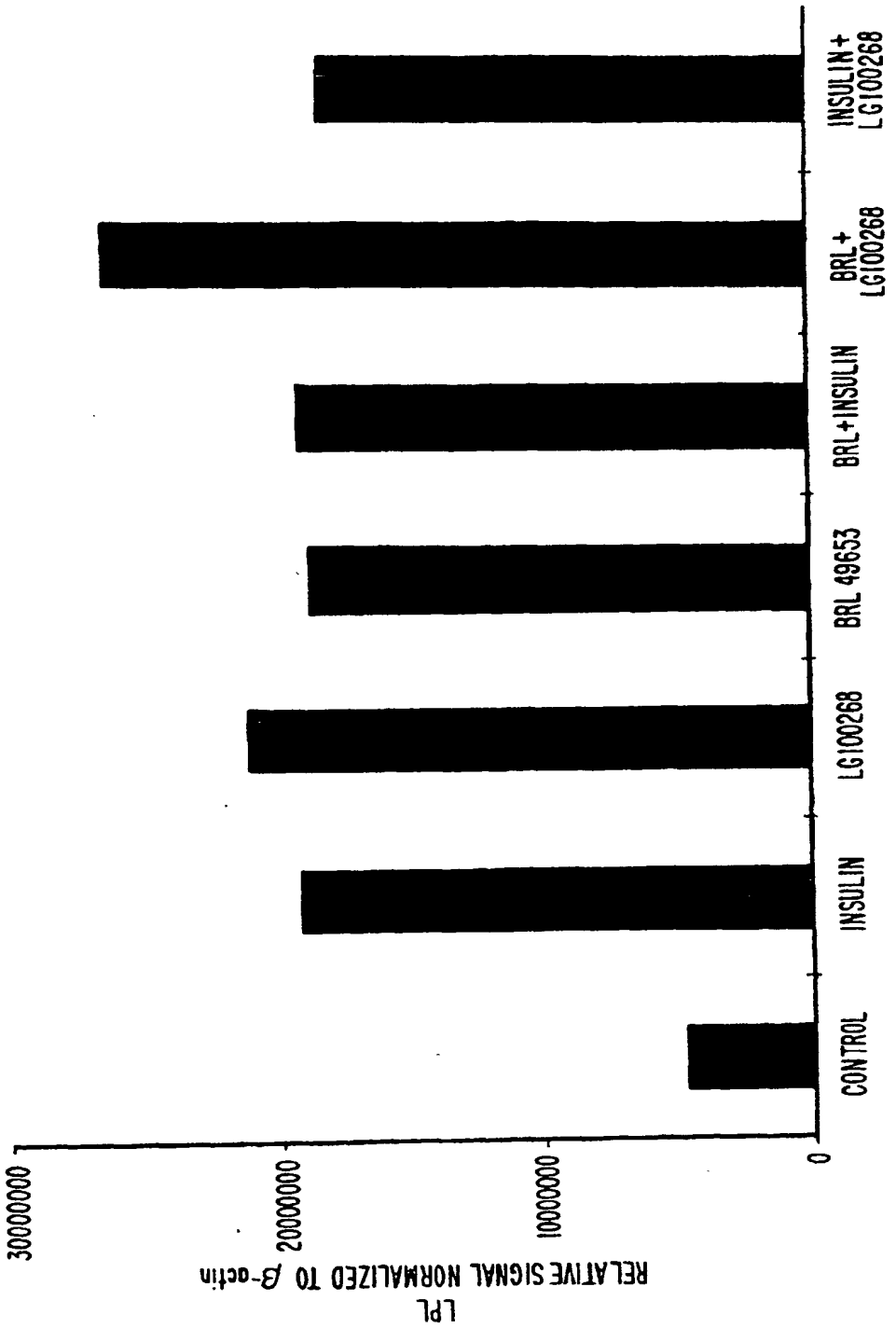

TRIGLYCERIDE (ug) NORMALIZED TO PROTEIN
0
2
4
6
8
10
FBS
BRL 49653
INSULIN
BRL+INSULIN
LDG1069
BRL+ LDG1069
INSULIN+ LDG1069
BRL+INSULIN + LDG 1069

FIG. 2a.


LPL
RELATIVE SIGNAL NORMALIZED TO β-actin
30000000
20000000
10000000
0
CONTROL
INSULIN
LG100268
BRL 49653
BRL+INSULIN
BRL+ LG100268
INSULIN+ LG100268

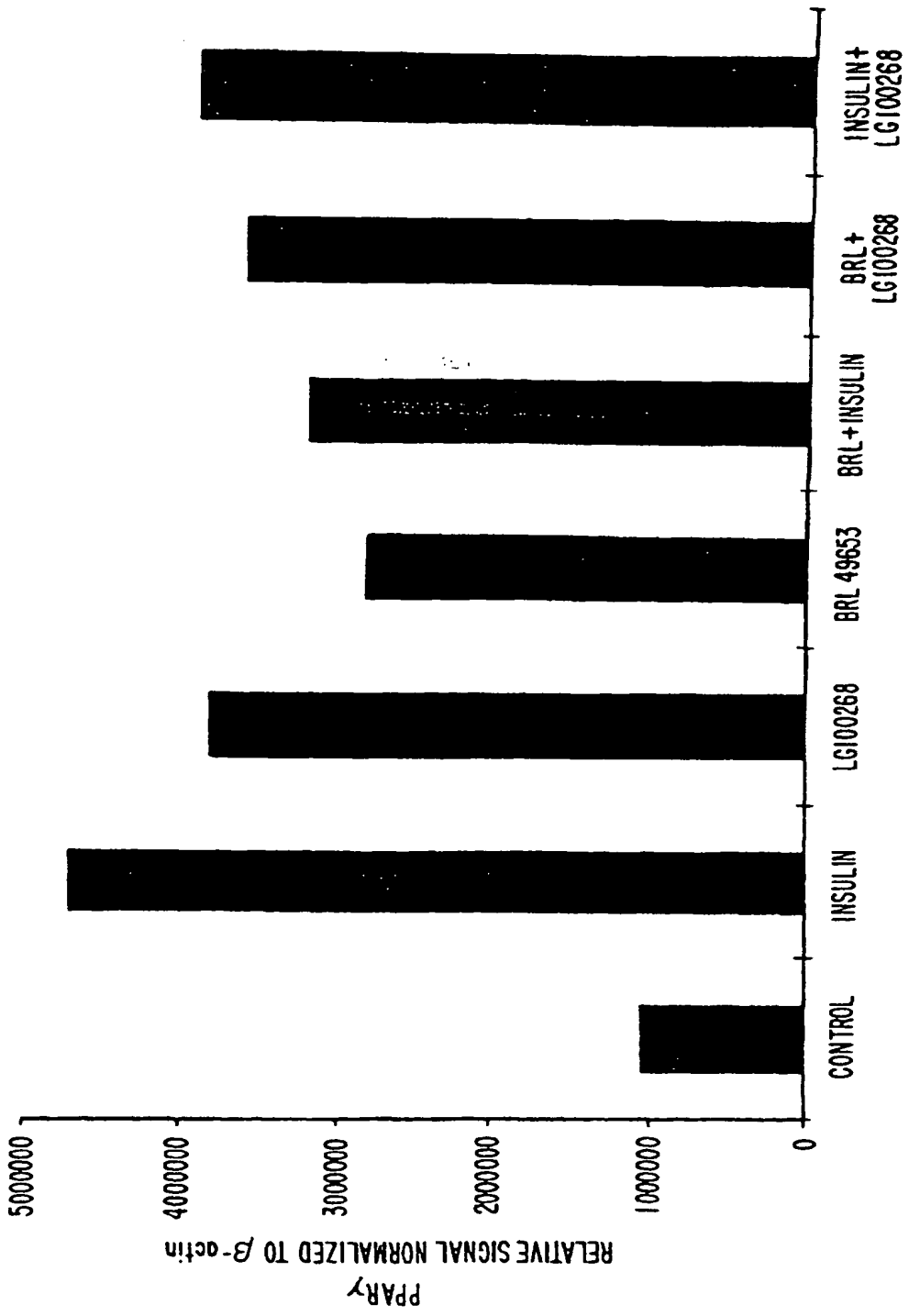
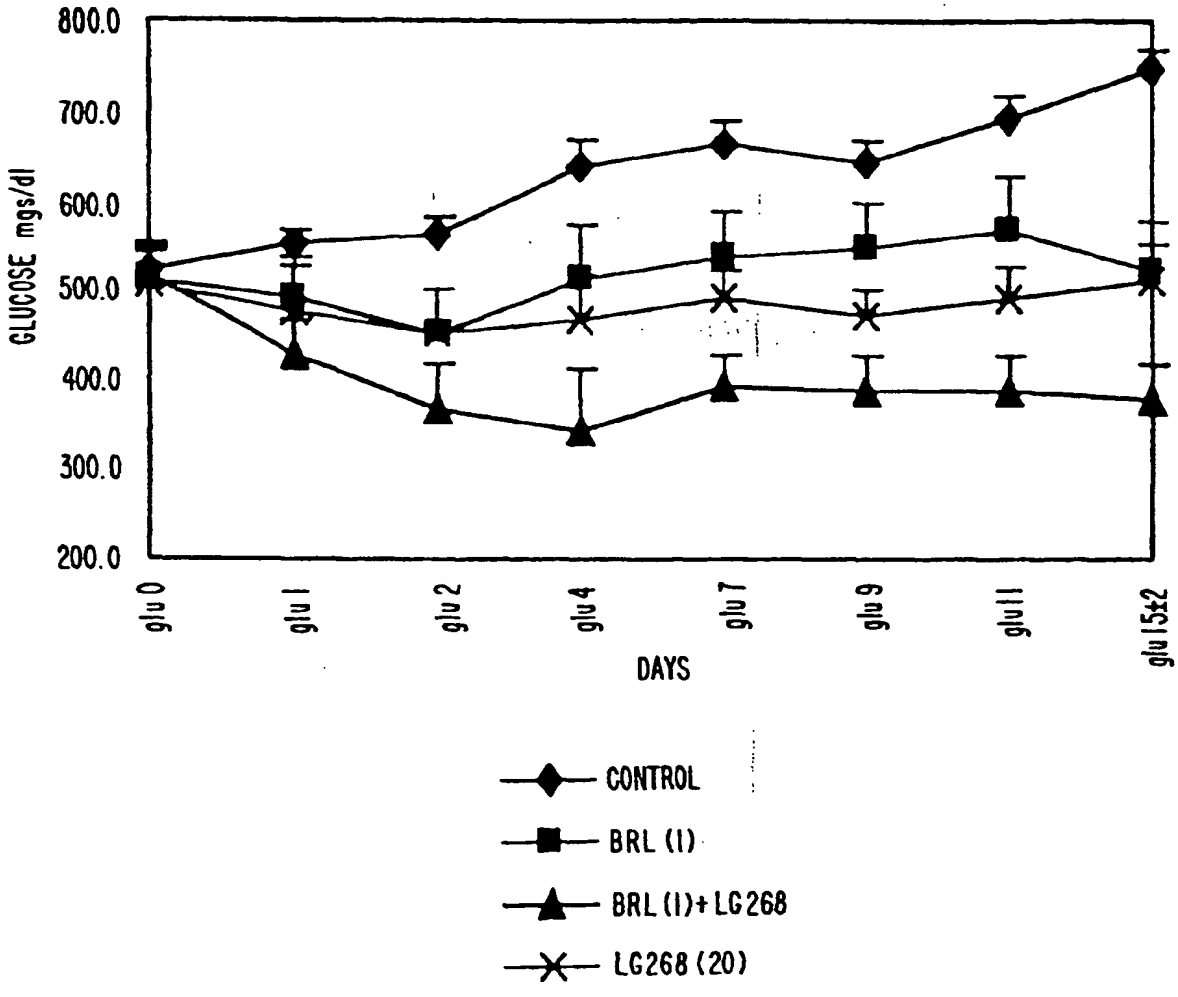

PPARγ
RELATIVE SIGNAL NORMALIZED TO β-actin
5000000
4000000
3000000
2000000
1000000
0
CONTROL
INSULIN
LG100268
BRL 49653
BRL+INSULIN
BRL+ LG100268
INSULIN+ LG100268

FIG. 2b.

FIG. 3.


800.0
700.0
600.0
500.0
400.0
300.0
200.0
GLUCOSE mgs/dl
glu 0
glu 1
glu 2
glu 4
glu 7
glu 9
glu 11
glu 15±2
DAYS
CONTROL
BRL (1)
BRL (1)+LG268
LG268 (20)

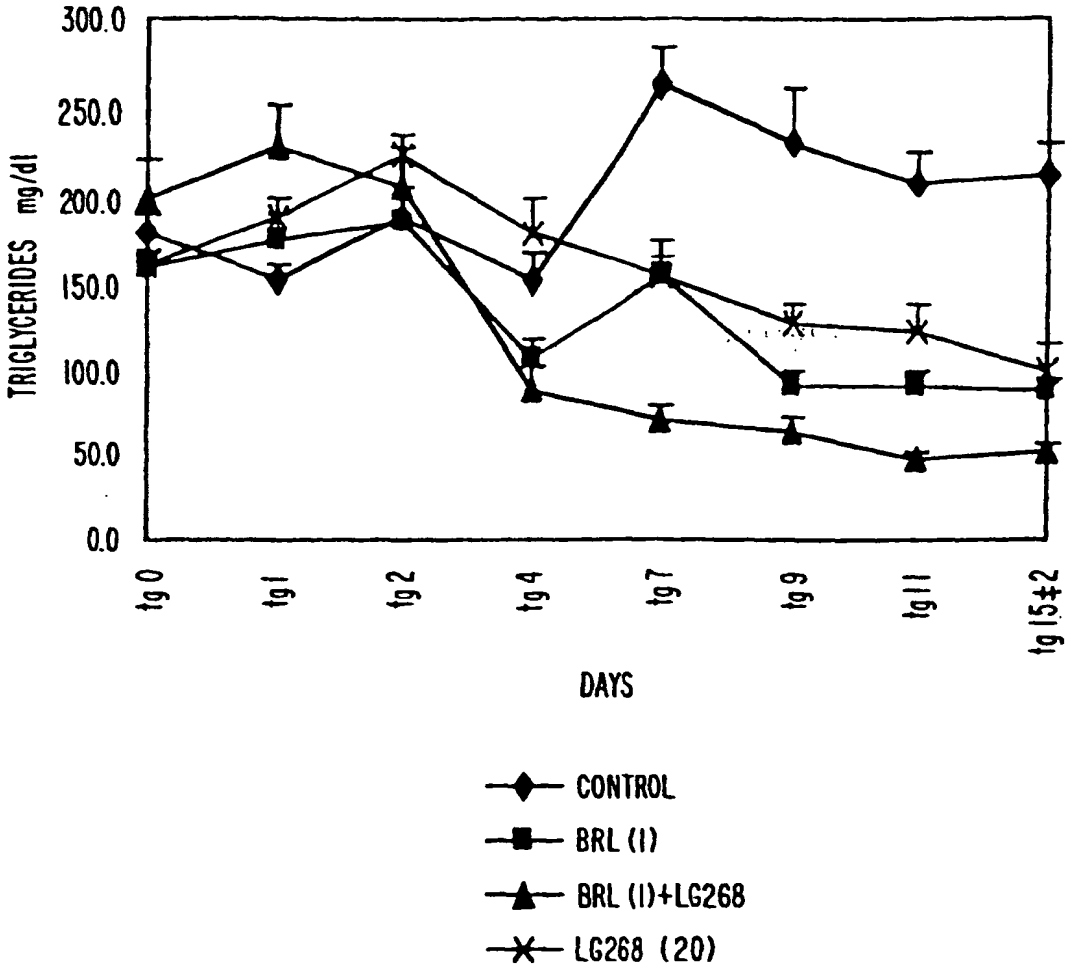

300.0
250.0
200.0
150.0
100.0
50.0
0.0
TRIGLYCERIDES mg/dl
tg 0
tg 1
tg 2
tg 4
tg 7
tg 9
tg 11
tg 15±2
DAYS
CONTROL
BRL (1)
BRL (1)+LG268
LG268 (20)

FIG. 4.

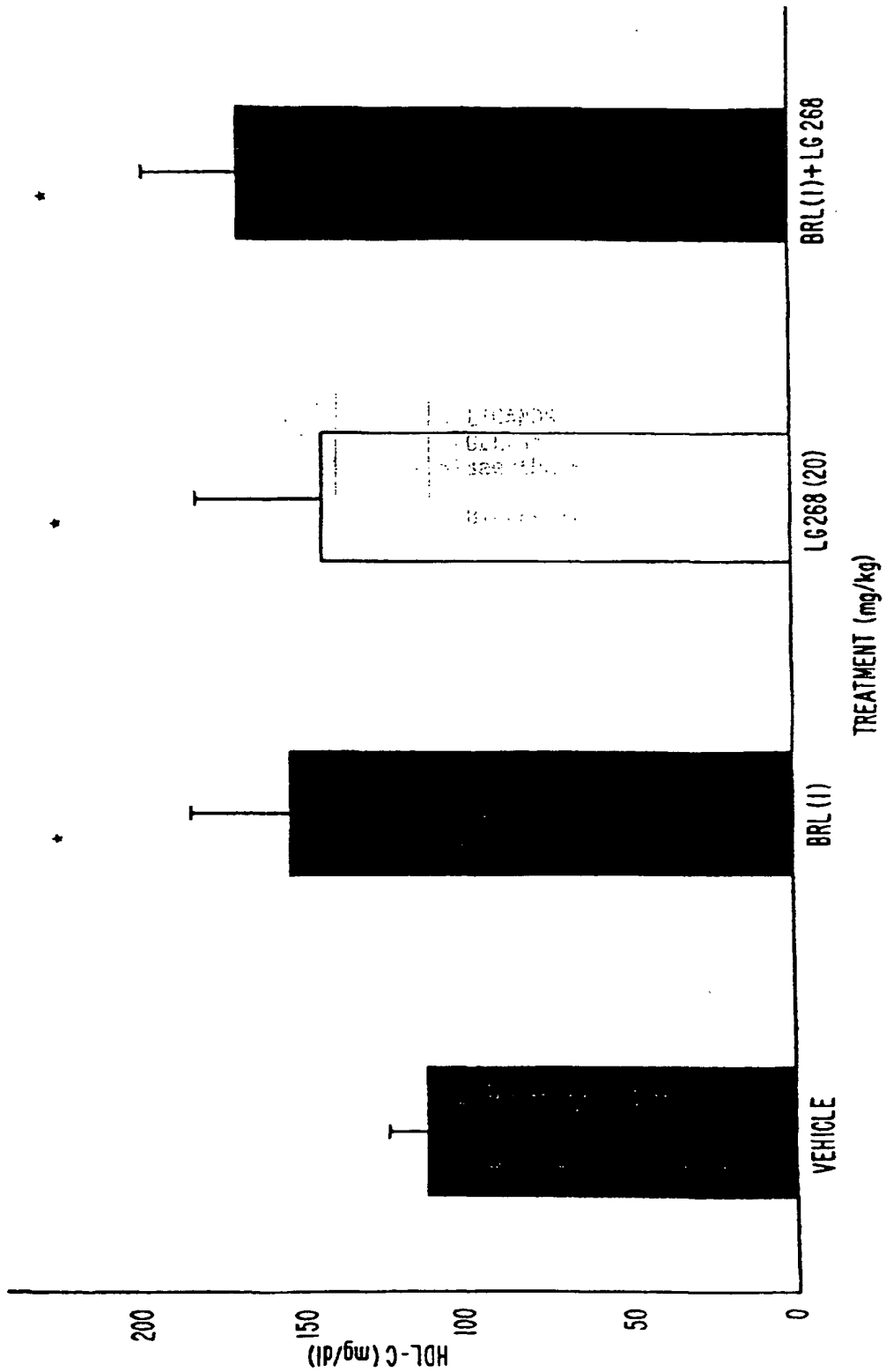

HDL-C (mg/dl)
0
50
100
150
200
VEHICLE
BRL (1)
LG268 (20)
BRL(1)+LG 268
TREATMENT (mg/kg)

FIG. 5.

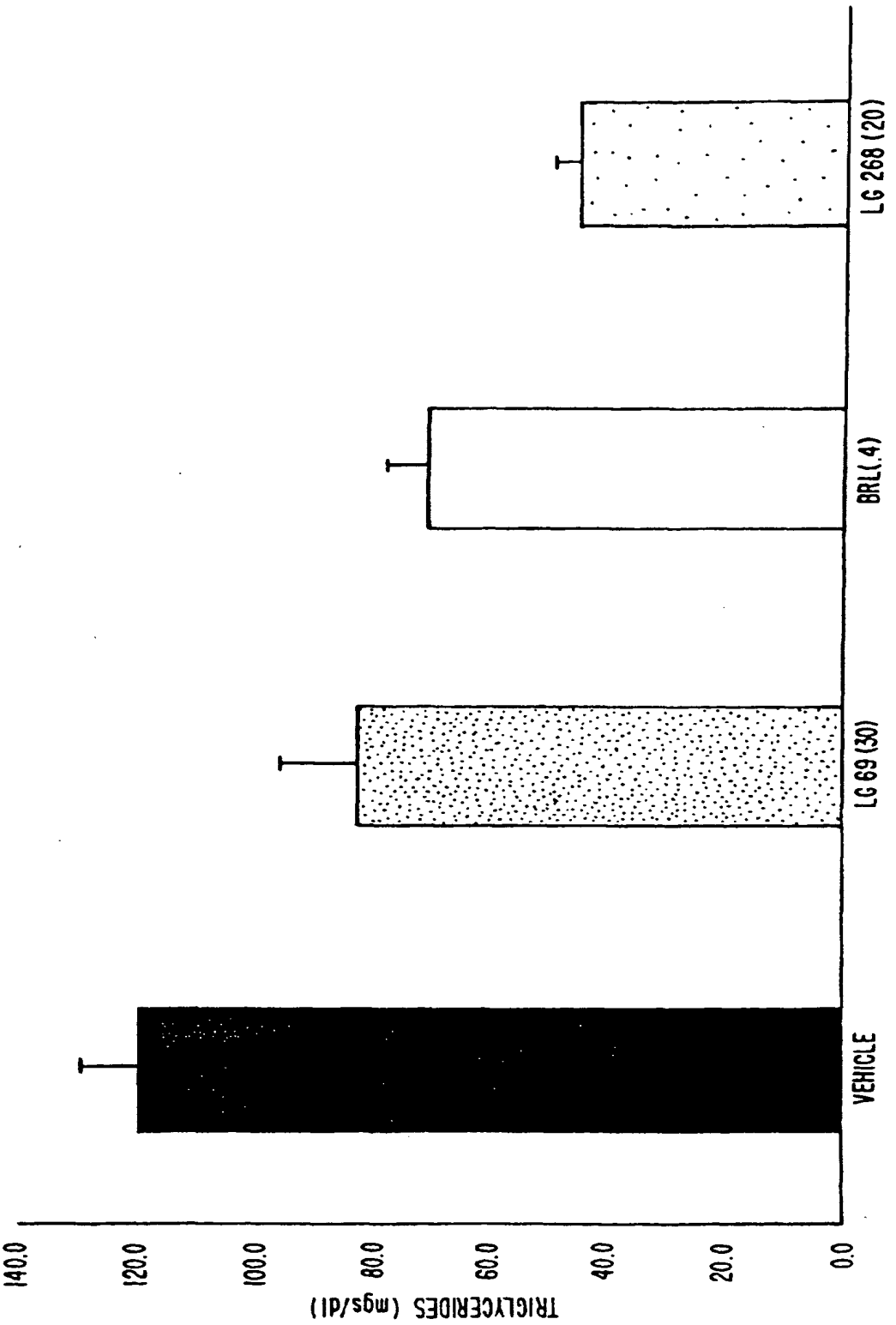

TRIGLYCERIDES (mgs/dl)
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
VEHICLE
LG 69 (30)
BRL(.4)
LG 268 (20)

FIG. 6.

FIG. 7.

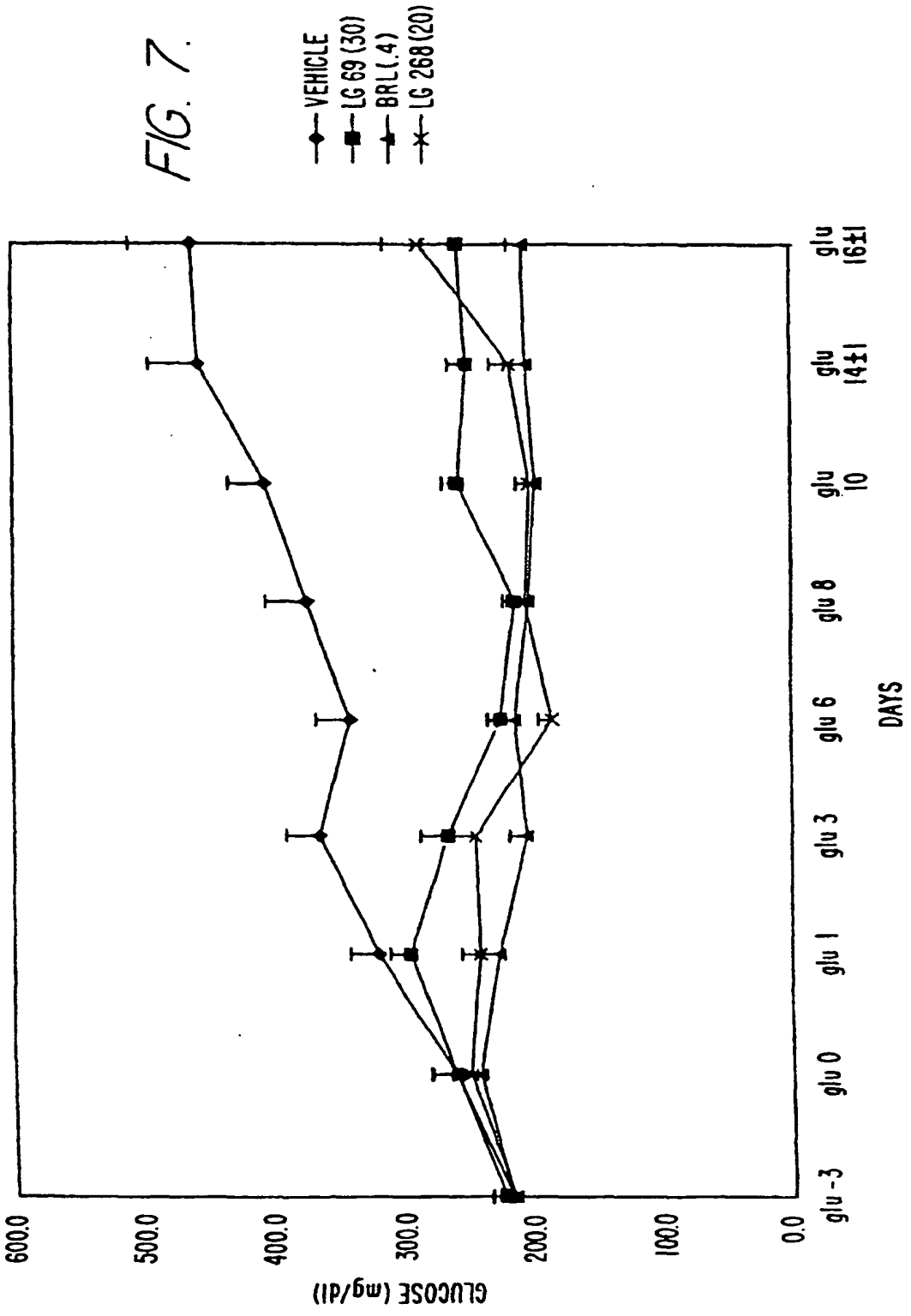
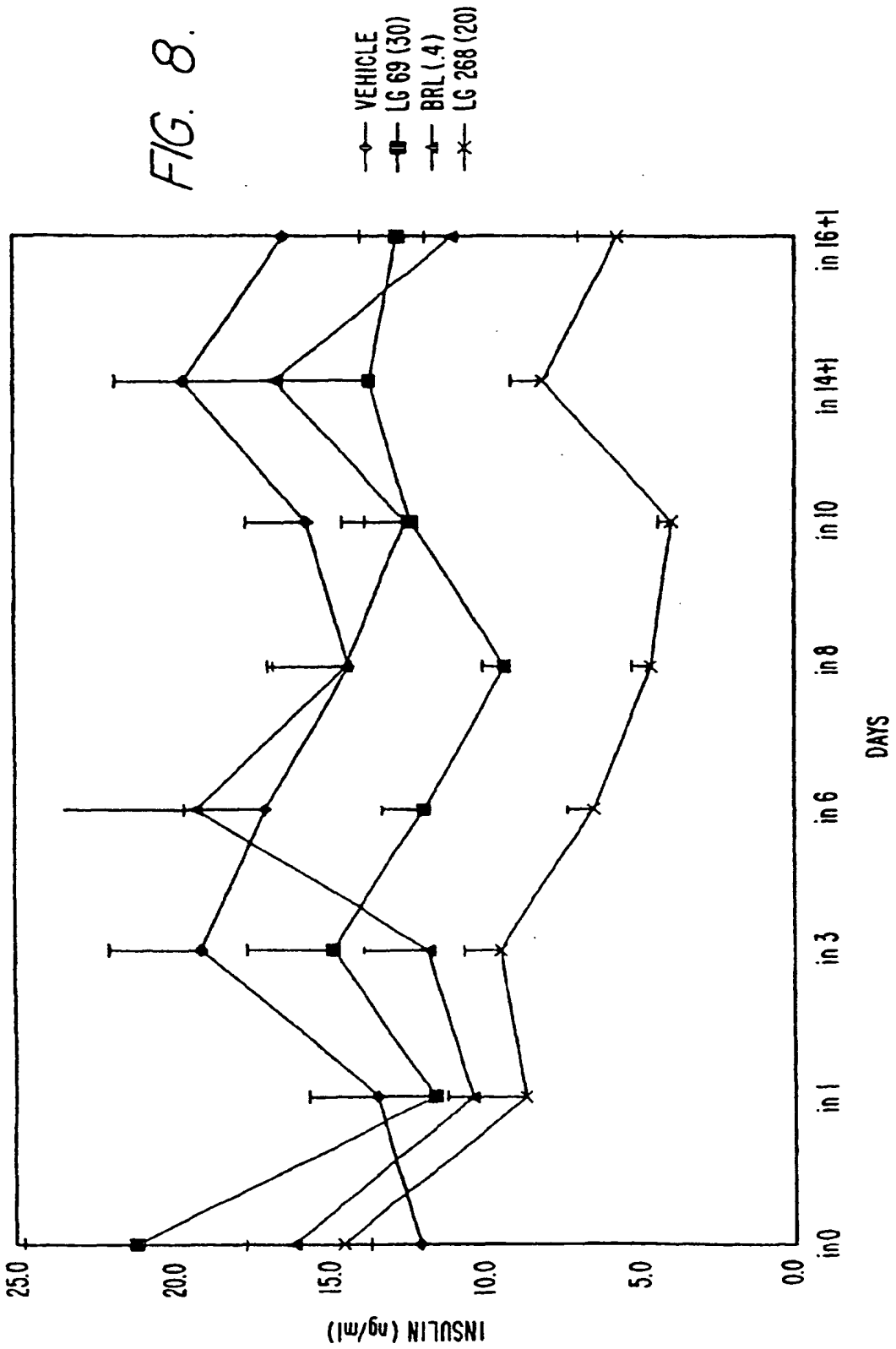

VEHICLE
LG 69 (30)
BRL(.4)
LG 268 (20)
GLUCOSE (mg/dl)
600.0
500.0
400.0
300.0
200.0
100.0
0.0
glu -3
glu 0
glu 1
glu 3
glu 6
glu 8
glu 10
glu 14±1
glu 16±1
DAYS

FIG. 8.


VEHICLE
LG 69 (30)
BRL (.4)
LG 268 (20)
INSULIN (ng/ml)
25.0
20.0
15.0
10.0
5.0
0.0
in 0
in 1
in 3
in 6
in 8
in 10
in 14+1
in 16+1
DAYS